

DRUG HYPERSENSITIVITIES: WHICH ROOM FOR BIOLOGICAL TESTS?

María L. Sanz, Pedro M. Gamboa (1), Carmen García-Avilés, Alain De Weck

SUMMARY: Drug allergic reactions frequently represent a serious diagnostic problem. In this paper we summarise the most relevant data published in recent years on the diagnostic reliability of the *in vitro* techniques in drug allergy diagnosis.

The lymphocyte transformation test (LTT) offers a sensitivity of 58% in the diagnosis of late allergic reactions to betalactams and 64.5% in the immediate allergic reactions. The basophil activation test and the antigen-specific sulphidoleukotriene determination have an acceptable diagnostic reliability in muscle relaxant drug-induced reactions and in betalactam allergy. BAT sensitivity in betalactam allergy was 50.7% and its specificity 93.3%, whereas CAP had a sensitivity of 36.7% and a specificity of 83.3%, and CAST, a sensitivity of 47.7% and a specificity of 83.3%. For NSAID hypersensitivity, BAT sensitivity was 63.3% and specificity 93.3%, CAST sensitivity was 38.3% and specificity 76.6%. BAT sensitivity in metamizol allergy was 42.3% and the specificity 100% and CAP was negative in all the 17 cases in which it was performed.

The joint use of BAT and CAP (specific IgE) allows diagnosis of 65.2% of the betalactam allergic patients with a specificity of 83.3%. The combined use of CAST and BAT in metamizol allergy detects 76% of the cases and 76.9% when associating the skin tests. In NSAID hypersensitivity, the joint use of BAT and CAST does not increase the diagnostic reliability of BAT alone.

BAT is a non-invasive useful technique in the *in vitro* diagnosis of betalactam and metamizol allergy, and NSAID hypersensitivity.

Key-words: Drug allergy - *In vitro* diagnosis - Biological test - Metamizol allergy - Betalactam allergy - NSAID hypersensitivity.

INTRODUCTION

Drug allergic reactions frequently represent a serious diagnostic problem. This is due to the potential risk of the *in vivo* methods on one hand, and on the other to the lack of *in vitro* tests or to their low reliability, especially for some drugs, such as NSAIDs.

In our environment, betalactam antibiotics are the most frequent cause of IgE-mediated reactions to drugs, followed by pyrazolones (1). There is a possibility to determine specific IgE to betalactam antibiotics but up to now there are no *in vitro* diagnostic tests whose usefulness has been demonstrated in the diagnosis of pyrazolone allergy or NSAID hypersensitivity.

The first important handicap of **specific IgE determination** in the *in vitro* diagnosis of drug allergy is that it is available for a very limited panel of drugs only, such as penicillin, insulin, protamine and thiopental.

According to Blanca *et al.* (2), the sensitivity of CAP FEIA (Pharmacia) in betalactam allergy is 74%, the

best results are obtained with amoxicillin and benzylpenicilloyl (BPO) with a specificity of 96% and 100%. The same group studied a group of subjects presenting immediate allergic reactions to a penicillin derivative who were skin test negative but positive serum IgE antibodies. Out of 290 patients with immediate allergic reactions to a penicillin derivative, 40 (13.8%) were skin test negative and RAST positive (3).

The serum tryptase and histamine determinations are helpful in the diagnosis of anaphylactic shock and anaphylaxis, but serial measurements may be needed to confirm mast-cell participation in milder reactions (4,5).

A biological allergy test is a cellular *in vitro* assay where cells from a patient are stimulated by a putative allergen and a biological response observed. The antigen-specific *in vitro* stimulation reproduces to some extent the cellular activation conditions following *in vivo* contact with the drug, and offers theoretically specific information because it constitutes an *ex vivo* provocation test.

■ **Lymphocyte transformation test (LTT) in the diagnosis of late drug allergic reactions.** In late drug allergic reactions the lymphocyte transformation test (LTT) detects cellular proliferation or blas-

Department of Allergology and Clinical Immunology. University Clinic of Navarra. School of Medicine. University of Navarra - 31080 Pamplona (Spain).

(1) Allergy Service. Hospital Basurto. Bilbao. Spain.

togenesis after incubation of the lymphomonocytic cells with the suspected drug. The lymphocyte transformation test (LTT) measures *in vitro* the proliferation of T cells to a drug from which one may conclude that a previous *in vivo* reaction has been due to specific sensitization. This LTT phenomenon has been confirmed by the generation of drug-specific T-cell clones and the finding that some drugs can directly interact with the T-cell receptors, without previous metabolism or the need to bind to protein carriers. The main advantage of this test is its applicability with many different drugs in different types of immune reactions, as drug-specific T cell are almost always involved in drug hypersensitivity reactions. Its main disadvantages are that an *in vitro* proliferation of T cells to a drug is difficult to relate to the clinical situation and that the test per se is rather cumbersome and technically demanding. In addition, its sensitivity is limited (for betalactam allergy it is in the range of 60-70%). Consequently, drug hypersensitivity diagnosis needs to rely on a combination of history and different tests, as none of the tests available has per se a sufficient sensitivity when used alone. Within these limitations, the LTT has proven to be a useful test for the diagnosis of drug hypersensitivity reactions and has helped to better understand these reactions (6).

According to Luque *et al.* (7), the overall sensitivity of LTT in the allergic patients was 62%, but, when analyzed separately, sensitivity was 64.5% for the immediate reacting group and 57.9% for the non-immediate group. The LTT specificity was 92.8%. These authors conclude that the LTT should be considered a useful *in vitro* diagnostic tool to identify subjects allergic to penicillins, especially patients with non-immediate reactions where the LTT has a better diagnostic value than skin tests. Interestingly, positive T-cell proliferative responses can be observed 10 or more years after the occurrence of the reaction without further exposure to the drug.

Further work on the simplification of this test and systematic evaluation of its sensitivity and specificity in some main groups of drugs are necessary to make this test more widely available.

■ **T cell activation** can be detected by flow cytometry analysis after incubation with the specific antigen. After seven days of culture, activated cells can be detected by the presence of the CD25 molecule (interleukin-2 receptor) on CD3+ T-cells (8). With CD69 staining, T-lymphocyte activation can be detected from the first 24 h of co-culture with the

antigen (9). Up to now, however, there are no validated results with these techniques in drug allergy.

■ **Basophil activation markers and antigen-specific sulphidoleukotriene production in the *in vitro* diagnosis of drug allergy or hypersensitivity**

New methods, like the basophil surface marker for activation CD63 are replacing old ones like histamine release for immediate type hypersensitivity reactions.

In the late 1990s basophils were shown to upregulate the expression of a number of surface proteins (eg, CD45, CD63, CD69 and CD203c) when activated by allergen (10). CD63 molecule is a 53 Kda tetraspan granular protein that is expressed not only on the basophil granules and on their surface when it is activated, but also on monocytes, macrophages and platelets. The expression of this marker is correlated with degranulation, which makes of this molecule a perfect marker of the basophil activation (11).

This molecule can be detected by flowcytometry, using two monoclonal antibodies: anti CD63 PE and anti IgE FITC.

During the last five years, an increasing number of studies have demonstrated that flowcytometric quantification of *in vitro* basophil activation can be quite efficient and reliable tool to measure IgE-dependent allergen specific response in allergic patients (12).

■ Moneret *et al.* (13) studied 50 patients with **muscle relaxant drug-induced allergic reactions** and flow cytometry using CD63 and CCR3 and found a sensitivity of 54%. Abuaf *et al.* (14) studied the basophil activation by muscle relaxant drugs using the "BASIC" assay, which measures the intensity modifications of the fluorescence opposed to the percentage of positive cells. The authors proved a characteristic dose-response curve, but indicate that high concentration of drugs can act as unspecific basophil activators.

Sudheer *et al.* (15) examined flow cytometric analysis of the expression on the cell surface of the basophil activation markers CD63 and CD203c and measured histamine release in 21 patients who were referred with possible peri-operative anaphylaxis. The sensitivity of CD63, CD203c, basophil histamine release and skin prick for the muscle relaxants was found to be 79%, 36%, 36% and 64%, respectively; the specificity was found to be 100%.

We studied the diagnostic reliability of this technique in the *in vitro* diagnosis of allergy to different drugs, such as betalactam antibiotics and metamizol, as well as of NSAID hypersensitivity.

Betalactam allergic patients

We studied a group of 92 patients with betalactam antibiotic allergy who had presented anaphylaxis or urticaria-angioedema immediately after administration of betalactam antibiotics; 69 of them had positive skin test at least to one of the allergens tested: MDM, PPL, penicillin, ampicillin, amoxicillin and the cephalosporins involved in the reaction. The second group included 23 patients allergic to betalactams with negative skin tests to betalactams. Five of them had positive CAP, 12 had negative CAP to betalactams but positive provocation tests, and 6 had negative CAP but more than two episodes of anaphylaxis/urticaria after amoxicillin administration.

As control group, 30 non allergic patients (13 males and 17 females) (52.5 years $\pm$ 14.9) were chosen. All of them had negative skin tests to betalactams and tolerated their administration.

Metamizol allergic patients

We include a group of 26 metamizol allergic patients and 30 healthy controls. Out of the 26 patients, 14 had presented anaphylaxis, 12 urticaria-angioedema immediately (less than one hour) after the exclusive administration of Metamizol alone. NSAID hypersensitivity was ruled out in all the patients by provocation test to ASA and diclofenac. The skin tests were positive in 13 patients (50%). The 30 healthy controls had negative skin tests and oral provocation test to metamizol and aspirin.

Patients with NSAID hypersensitivity

Sixty NSAID hypersensitive patients (38 with cutaneous, 20 with airway and 2 with cutaneous and airway symptoms) and 30 control patients (15 extrinsic asthmatics) were selected. Their hypersensitivity was confirmed by documented history indicating at least two clinical episodes to two or more different NSAIDs or by positive oral provocation challenge. Isolated buffy coat leukocytes were stimulated *in vitro* with aspirin, paracetamol, metamizol, diclofenac, and naproxen. The percentage of activated basophils was evaluated by an anti-CD63 antibody. As control group, 30 healthy controls without history and who tolerated NSAIDs were included.

METHODS

Skin tests

The usual procedure was used for skin tests (16), as previously described (17-20).

■ **Specific IgE determination** : specific IgE was determined by CAP (Pharmacia Uppsala, Sweden) in the cases of betalactam allergy with penicillin G, penicillin V, ampicillin and amoxicillin, and in 17 cases of patients with metamizol allergy. Results equal to or greater than 0.35 kU/l were considered positive.

■ **Basophil activation test and Sulphidoleukotrien determination**: Sulphidoleukotriene production was determined by CAST-ELISA (Bühlmann laboratories, Allschwil, Switzerland) in all the cases and controls. BAT was performed as described in detail elsewhere (17, 19).

■ **Statistical analysis** : The data were analysed by a SPSS 10.0 program. The data were expressed in means $\pm$ standard deviation. The reproducibility of BAT was analysed by the intraclass correlation coefficient. Values of $p < 0.05$ were considered significant. To obtain the optimal cut off point of the BAT and CAST we applied ROC curves (Receiver operating characteristic).

RESULTS

BAT, CAST and CAP in betalactam allergic patients

■ **Patients with positive skin tests**: Sensitivity of BAT was 50.7% (35/69) and its specificity 93.3% (28/30) for a cut off point of basophil activation greater than 5% and a stimulation index (SI) greater than 2 (SI= activation with the drug/basal activation). Specific IgE determination by CAP (Pharmacia) showed a sensitivity of 36.7% and a specificity of 83.3%. CAST presented a sensitivity of 47.7% and a specificity of 83.3%. The combined use of CAP (Pharmacia) and BAT allowed identification of 83.3%. The joint use of CAP (Pharmacia) and BAT allowed identification of 65.2% of the patients with a specificity of 83.3%.

The positive predictive value (PPV) in BAT was 19.4% and the negative predictive value (NPV) 98.4%. The PPV of CAP was 6.4% and the NPV 97.7%. For CAST the PPV was 8.1% and the NPV was 98.1%.

■ **Patients with negative skin tests**: Out of the 23 definitely allergic patients to betalactam antibiotics

but with negative skin tests, BAT was positive in 39.1% of the cases with a specificity of 93.3%. CAST was positive in 22.7% of the cases with a specificity of 83.3% and CAP showed a sensitivity of 21.7% with a specificity of 86.7% (table I). The association of the three techniques allows diagnosis of 60.9% of this group of patients with a specificity of 70%.

BAT, CAST and CAP in metamizol allergy

In 11 of the 26 patients, BAT was positive whereas all the controls were negative. Sensitivity of the technique was 42.3% and the specificity was 100% for a cut off point of basophil activation greater than 5% and a stimulation index (SI) greater than 2. Assuming a prevalence similar to the one of the betalactams (1%), the positive predictive value of BAT is 100% and the negative predictive value 99.4%. CAP was negative in the 17 cases tested and CAST offers a SE of 52% and a SP of 90%.

The association of BAT and CAST allows detection of 76% of the cases, whereas the combined use of BAT, CAST and skin tests hardly increases the diagnostic reliability (76.9%).

BAT and CAST in NSAID hypersensitivity

In the BAT test, aspirin as allergen showed a sensitivity of 43.3 %, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 99.4%. For the other NSAIDs, the sensitivity and specificity values were respectively for paracetamol 11.7% and 100%, for metamizol 15% and 100%, for diclofenac 43.3% and 93.3 %. When considering the four NSAID, the global sensitivity raised to 63.3% while specificity 93.3%. If the number of tests is to be limited for practical reasons, the combination of ASA and diclofenac at two concentrations yields a sensitivity of 58.3% and a specificity of 93.3%.

CAST showed a SE of 38.3% and a SP of 76.7% (global values with aspirin, paracetamol, metamizol and diclofenac).

The association of BAT and CAST in this group does not increase the diagnostic reliability of BAT.

Reproducibility

The reproducibility of the technique is very high, its intraassay variation coefficient being 0.89 ($p < 0.0001$) for betalactams, 0.99 ($p < 0.0001$) for Metamizol and 0.92 ($p < 0.0001$) for NSAIDs.

DISCUSSION

The *in vitro* diagnosis of drug allergy is a difficult problem that has not been satisfactorily solved up to now. In the diagnosis of late allergic reactions to drugs, the lymphocyte transformation test (LTT) shows a sensitivity of 60 and 70% for betalactam antibiotics. Provided that a correct clinical diagnosis is made, this test can be a useful tool in this low prevalence group of patients with specific late reactions to betalactam. But proliferating cells may not be the effector cells and a positive LTT could only signal a previous contact with the drug, immune memory or proliferation of regulatory T-cells. Indeed, as T-specific memory is necessary for the production of antibodies by B-lymphocytes, cell proliferation is not specific of delayed-type hypersensitivity. Specific cytotoxicity assays would be necessary to confirm that proliferating cells are the effector cells. The sensitivity of the LTT for the detection of drug sensitization could be improved up to 92% by the measurement of released interleukin-5. The expression and secretion of other cytokines such as IFN-gamma and IL-10 was less consistently and had a diagnostic sensitivity of 36 and 50%, respectively. Microarrays are a promising new technical platform to look for better markers which can be used as a read out in the LTT and other similar assays and to study pharmacological interactions between drugs including cytokines such as interferons and the immune system (21).

Regarding the diagnosis of immediate type allergic reactions to betalactams, previous studies suggest that *in vivo* tests, such as skin tests to penicillin, benzilpenicillin G and penicillin minor determinants (MDM) could identify up to 95% of the patients with betalactam allergy (22) but in recent years, amoxicillin has to be added to the skin test allergens used (23). According to some authors (24) negative skin tests to PPL, benzylpenicillin G and MDM allow a safe administration of penicillin. Nevertheless, at least 5% of the patients with positive clinical history and negative skin tests have a positive result in the provocation test (23).

In vitro tests that measure specific IgE to betalactams do not confirm or rule out the diagnosis of this allergy in a high percentage of cases (25).

Flow cytometry and particularly CD63 detection in activated cells has been observed to be useful in the diagnosis of various allergic pathologies (26-29). Its high reliability in the *in vitro* diagnosis of IgE-mediated allergy to *Der p*, *Lol p* (30), cypress pollen (31), latex (32) and food allergens (33) has been reported.

In 50% of the patients presenting betalactam allergy and positive skin test we observed a significant baso-

phil activation after incubation of the cells with these antibiotics. In patients with betalactam allergy and negative skin test, BAT shows a greater diagnostic reliability than CAP and CAST. The association of BAT with specific IgE determination in allergy to betalactam antibiotics allows diagnosis of 65% of the cases. Other authors report too the combined use of BAT and CAP/RAST with similar results to ours (29). When we associate the three *in vitro* techniques (BAT, CAST and CAP), the tests allow detection of 60.9% in this group of patients with a specificity of 70% (34).

In the group of patients with metamizol allergy, BAT presents a specificity of 100% and a sensitivity of 42.3% (18). IgE-mediated reactions to pirazolones are the second cause of drug allergy in our environment, and is frequently rather severe. Up to now, the only tests available were the skin and the provocation tests, which entail a risk for the patient. BAT and CAST are the only *in vitro* tests that have proven their usefulness in this pathology and that allow to avoid performance of provocation tests in a high percentage of cases. Association of BAT and CAST detects 76% of the patients with metamizol allergy.

No other techniques allow detection of a sensitisation to Metamizol *in vitro*, or to many other drugs such as heparins, antibiotics, omeprazol, corticosteroids, muscle relaxants, etc.

There are few studies in the literature that validate the reliability of cellular *in vitro* tests in drug allergy; among them the ones performed by us and other authors (17, 18, 29, 35-37) report the sensitivity and specificity of BAT, as well as its reliability compared with other techniques such as CAP and sulfidoleukotriene determination by CAST.

Our results confirm that BAT sensitivity is greater than CAP (specific IgE determination) in betalactam allergy.

In NSAIDs hypersensitive patients, BAT is an *in vitro* diagnostic test with very high specificity and a sensitivity of 60–70%, well correlated with positive challen-

ge tests. As described here, it could be of definite use to the patients (20). It will enable with no risk and inconvenience to confirm the clinical diagnosis and to avoid provocation, in a sizeable number of cases. When negative, however, it does not allow to exclude NSAID hypersensitivity and provocation challenge remains necessary, if clinically indicated.

BAT shows a high reproducibility and the best sensitivity and specificity values of all three tests. In any case, we can affirm that the association of BAT and CAST in betalactam and metamizol allergy and the use of BAT exclusively in hypersensitivity to NSAID are useful tools in the *in vitro* diagnosis of these pathologies and allow avoidance of provocation test in a far from negligible number of cases.

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		BL allergy with +SKT N=69	BL allergy with -SKT N=23	Metamizol allergy N=26	NSAID hypersensitivity N=60
BAT	SE%	50.7	39.1	42.3	63.3
	SP%	93.3	93.3	100	93.3
CAST	SE%	47.7	22.7	52	38.3
	SP%	83.3	83.3	90	76.7
CAP	SE%	36.7	21.7	0	-
	SP%	83.3	86.7	-	-

Table 1: Sensitivity and specificity of BAT, CAP and CAST in betalactam and metamizol allergy, and in NSAID hypersensitivity.

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